PLANT SCIENCE

The Vavilov Institute's (VIR) contribution to the survey and study of *Vavilovia formosa* (Fabaceae)

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Abstract

In the 1960s–1980s, the Vavilov Institute of Plant Industry, known worldwide as VIR, organized a substantial series of studies dedicated to a Caucasian endemic, the Pliocene relic *Vavilovia formosa* (Fabeae: Fabaceae). Those investigations are little known to the scientific community, although, in fact, the priority right for integrated research into this plant belongs to VIR. As a result of the cycle of studies, the first ideas about the biology and ecology of the species, the manner of its reproduction, its intraspecific diversity, the degree of crossability with other members of the tribe, its karyotype and anatomy were developed. The plants of *V. formosa* are not reproduced *ex situ* and their germplasm is absent in the world's gene banks. Therefore, collecting *V. formosa* plants in their hard-to-reach habitats in the mountains is always crucial for research purposes, so a number of collecting expeditions were launched to the Greater Caucasus. The data thus obtained are discussed in the context of modern achievements in world science regarding this species.

Keywords: *Vavilovia formosa*, Caucasian endemic, VIR, expeditions, collecting mission, ecological, anatomical, karyological, immunochemical, hybridological studies.

Introduction

Vavilovia formosa (Stev.) Fedorov, 1939 (tribe Fabeae Rchb. fam. Fabaceae Lindl.) is a perennial rhizomatous plant, growing exclusively in the high mountains of Western Asia and the Caucasus, the West Asian center of origin of the species, which is believed to be primary for the Fabeae tribe. The species is regarded as a relic of the Pliocene (Prima, 1974). Its monotypic and systematically isolated genus assigns the plant to the category of paleoendemic taxa (Kharadze, 1960).

In the Red Data Books of the USSR (1984) and the Russian Federation (2008), *V. formosa* is listed among the plants 'diminishing in numbers'. This definition is applicable to taxa whose numbers are reduced as a result of changes in the environment or habitat destruction. It is obvious that further impact of the factors that reduce the number of plants would quickly bring *V. formosa* into the category of endangered species.

V. formosa occurs exclusively in the subalpine and subnival zones, on rubbly gneissic, shale or tufa and pumice screes. The upper-alpine rock and scree vegetation, including *V. formosa*, is known to consist of pioneering plants which populated the land after the retreat of the glaciers. Having occupied finely-crushed stone screes, *V. formosa* with its rhizomes, along with other petrophytes, participated in the sodding process and contributed to the formation of montane grasslands. For this reason, some suggest that this kind of vegetation can be regarded as a temporary transitional phenomenon (Astamirova, 2011).

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This circumstance, along with poor access to the species' habitats and the small numbers of plants, made the search for its representatives difficult and often ineffective. Attempts to reproduce the species artificially were even less successful. Plants transferred from the highlands to lowlands failed to grow and subsequently died (Zhukovsky, 1971). All these facts make it clear why not a single gene bank holds the germplasm of *V. formosa*. Therefore, every effort of researchers to study this species compelled them to launch new searches for its plants in their natural habitats.

The analysis of the world's herbarium collections for the known localities of *V. formosa* made it possible to map 111 sites of its occurrence over the Main Caucasus Range and record *sensu stricto* the coordinates of the marginal points of its area of distribution in the Caucasus, together with its altitudinal boundaries, which varied from 2021 to 3500 MASL (Vishnyakova et al., 2015).

In addition to *V. formosa*, the Fabeae tribe incorporates four more plant genera, including such economically important species as *Pisum sativum* L., *Lens culinaris* Mill., and representatives of the genera *Vicia* L. and *Lathyrus* L. For a long time, the taxonomic status of *V. formosa*, its relationships with the other members of the same tribe, and its place in the tribe's phylogeny remained debatable (see Makasheva, Drozd, Adamova and Golubev, 1973; Mikic et al., 2010). In view of this, with the development of modern biotechnologies the interest in this relict plant species has grown considerably. *V. formosa* has become the object of molecular-genetic and molecular-phylogenetic research (Mikic et al., 2010; Oskoueiyan et al., 2010; Schaefer et al., 2012). Impressive progress was made in studying the soil microbiota of the species: the knowledge of its nature provided additional data on Fabeae phylogenesis and clarified the evolutional issues of the symbiotic links between plants and microorganisms (Safronova et al., 2014, 2015).

In recent years, some reassuring information has been collected about the successful maintenance of *V. formosa* plants in botanical gardens and in vitro (Ochatt and Conreux, 2016; Akopian et al., 2019), which would in its turn promote further research into this still insufficiently studied plant.

However, the world community is hardly aware of the vast complex works conducted at VIR in the 1960s–1980s with the aim of searching for and studying *V. formosa*. A number of plant explorations launched by VIR succeeded in collecting seeds and whole plants of *V. formosa*, thus initiating morphological, karyological, anatomical, hybridological and immunochemical research on this rare species at the Institute's departments and laboratories (Abramova, 1971; Makasheva, Drozd, Adamova and Golubev, 1973; Petrova, 1973; Tarlakovskaya, 1975; Makasheva, 1979; Tarlakovskaya, Gavrilyuk and Makasheva, 1979; Golubev, 1990). Indeed, VIR may claim a priority right to the complex study of *V. formosa.* Such research efforts helped to gain the first ideas of the species' biology and ecology, understand its reproductive pattern, throw light on its intraspecific diversity, identify the level of its crossability with other members of the same tribe, visualize its karyotype, anatomy of some vegetative parts, etc.

The purpose of this paper is to review the data obtained by VIR's scientists on *V. formosa* in the 1960s–1980s, using the ways and means of that time, and compare them briefly with the data of recent years, reported by various laboratories around the world.

Collecting missions and discoveries

In the 1960s, systemic research into the flora and vegetation of rocks and screes was initiated as part of an intensive complex analysis of highland plants. It provided the impulse to search for *V. formosa* (Astamirova, Abdurzakova, Magomadova and Umaeva, 2011).

In 1960, the first collecting mission was organized by VIR to search for plants belonging to this species, which was recognized at that time by the Institute as *Pisum formosum* (Stev.) Alef. under the common name of 'perennial pea' (Makasheva, Drozd, Adamova and Golubev, 1973). The collecting team departed to the Lateral Caucasus Range, in the upper reach of the Kuban River, where the first samples of the then-little-known species had been collected in 1900 on the peaks of Elbashi and Bgazyrty-Dippurt by Numa Desulavi, a renowned plant collector whose herbarium specimens are still preserved in a number of herbaria over the world (LE, K, TGM). However, after a painstaking exploration of the finely crushed stone screes on those mountains, the team found out that the populations of the species had disappeared. Nevertheless, their search proved successful a little to the east: on the peaks of Elmez-tyube and Sadyrlyar-bashi. Some of the plants were placed under isolators. The next collecting team (one month later) observed the absence of pod setting and, with a lapse of time, abscission of isolated seed buds, which attested to the need for cross-pollination and the absolute prevalence of vegetative reproduction. In 1963, the previous collecting sites were visited by another team of plant explorers. The three-year interval in explorations on the same peaks revealed the consequences of mighty landslides, which had destroyed half of the populations previously found on Mount Sadyrlyar-bashi (two out of four) (Makasheva, Drozd, Adamova and Golubev, 1973). Thus, the first notion about this plant's unstable habitats and its decreasing populations was obtained.

Several years later, VIR organized one more series of expeditions: repeated trips to the same sites of Stavropol Territory in 1970; to Armenia (Gegham Ridge), Dagestan and Azerbaijan in 1972; Stavropol Territory,

Fig. 1. A map with recorded sites of VIR's expeditions collecting *V. formosa* (1960–2011).

Armenia (Gegham Ridge) and Azerbaijan in 1973; Armenia (Gegham Ridge) and Dagestan in 1975; Armenia (Zangezur Mountains) and Dagestan in 1979; and a Soviet-British collection mission to Dagestan in 1989 (Makasheva, Drozd and Adamova, 1973; Golubev, 1990).

Two decades later, in 2010, one more collecting team visited the above-mentioned sites in Stavropol Territory (upper Kuban River), and thoroughly explored numerous stone screes on the peaks where populations of *V. formosa* had been discovered earlier. But the search proved futile. A change of the landscape was observed on the previously explored mountain tops: instead of rubbly screes, most of the mountain flanks were covered with steppified meadows, exposed to intensive grazing by livestock. On Elmez-tyube, the large-stone screes covering the mountain from top to bottom were partially overgrown by pines. Besides, hardly any snowfields were noticed on the peaks of the Sardyrlyar-bashi Ridge, where, according to the locals, snow had remained intact for practically the whole summer season, but now melted already at its beginning (Vishnyakova et al., 2013). It should be added that all the mentioned peaks are over 3000 m in height.

The latest findings in a search for *V. formosa* plants were made by a joint collecting mission of the Komarov Botanical Institute (BIN) and VIR in 2011, when the Dokuzparinsky District of Dagestan, including Mount Ragdan, was explored. The team assembled herbaria and collected only sporadic unripe seeds in late July/early August, when the plants were entering the flowering phase (Murtazaliev et al., 2012).

It should be mentioned that the previous missions had not always succeeded in collecting plant or seed samples of *V. formosa*.

It seems worthwhile to give two excerpts from the reports of the expeditions in Dagestan, in the vicinity of the village of Kurush. The first one, from 1972, reads as follows: "During September 11th–13th, the expedition examined the three sides of the scree over the pass of Shah-Dyuzi, the rise to which on horseback from the valley of Usukhtea took three hours. Climbing was difficult because of the deep snow that had fallen the day before. The members of the expedition overexerted themselves all three days and returned to Kurush in the dark. The entire daylight time was engaged in the search for wild peas. It was not possible to find them."

(Report…, 1972–1973). Three years later, the outcome was different: "Having surveyed the scree of the mountains Shalbuzdag and Tfan and the pass of Shah-Dyuzi, on Mount Ragdan and in the vicinity of the village of Kurush, several populations were found that served as material for the collection and research directly in situ." (Report…, 1975).

In total, during the period from 1960 through 2011, VIR conducted and participated in no fewer than 16 collecting missions dedicated to the search for *V. formosa*. Most of these endeavors resulted in adding plant materials and herbarium specimens to the Institute's holdings (Fig. 1).

Environmental, anatomical and karyological data

The delivered plants and seeds, collected in 1970–1980, were distributed to various stakeholders over the USSR, including different research institutions, botanical gardens and VIR's experiment stations, and grown under artificial climate conditions at VIR. Despite the efforts taken, the plants did not endure long cultivation and died. The crucial conditions ensuring successful plant growth in natural habitats were the absence of overheating, continuous watering (provided in nature by melting glaciers), sufficient aeration of the substrate, and low concentrations of minerals in the soil (Makasheva, Drozd, Adamova and Golubev, 1973). These environmental features, favorable for *V. formosa*, were confirmed by anatomical studies conducted at the Institute. Observations noted the presence of long horizontal underground stems (rhizomes), which produce overground stems. Unlike the latter ones, underground stems bear buds and secondary roots, which serve as tools facilitating the life of plants on a scree. The root and the underground stem were found to have cavernous vessels, testifying to the high intensity of the plant's water supply. The presence of underground stems, constantly existing in a damp environment due to the meltwater, prevents the plant from developing a deep root system. The thin cuticles on small thick leaves, presence of dense mesophyll tissue, absence of pubescence, and the numerous stomata on both sides of the leaf testify to the plant's demand for intensive transpiration to mitigate overheating (Petrova, 1973).

The karyotype of the species was studied for the first time (Abramova, 1971). The number of chromosomes in somatic tissue cells and the general structure of karyotypes were found to be identical (2n = 14) in the collected plants and the earlier studied *P. sativum* (Levitsky, 1930). Their common features were evident in karyotype asymmetry, prevalence of acrocentric chromosomes, and a gradual transition in their size. Each of the two species had two satellite chromosomes. Differences between the

Fig. 2. Metaphase plate of *Pisum formosum* (Stev.) Boiss. (reprinted from Abramova, 1971).

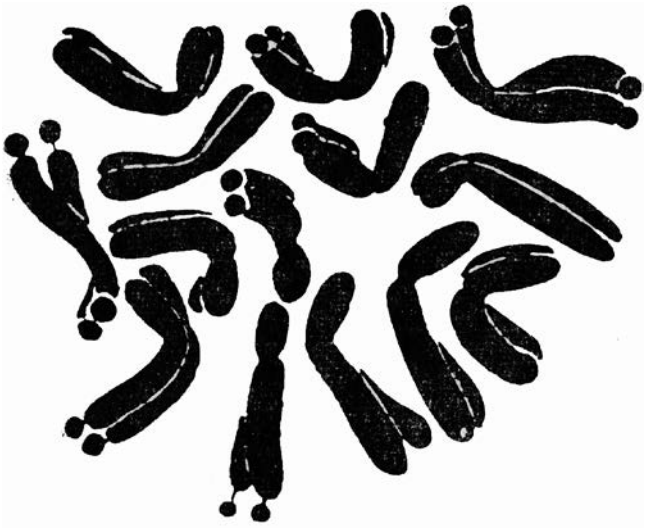

Fig. 3. Metaphase plate of *Pisum sativum* L. (Levitsky, 1930).

karyotypes were less obvious, but demonstrated stability. They were expressed in different numbers of acrocentric chromosomes and in the position of satellites within satellite chromosomes. In *P. formosum* one pair of acrocentric chromosomes was observed; in *P. sativum*—two pairs. In the satellite chromosomes of *P. formosum,* satellites appear on the shorter arm; in *P. sativum*—on the longer one (Fig. 2, 3). The author of this paper has not presented any measurements of the chromosomes, be-

cause she compared the studied karyotype with the one analyzed earlier by another researcher. Meanwhile, the differences in chromosome morphology observed between the two karyotypes were obvious and testified to a certain isolation of *P. formosum* (Abramova, 1971).

Intergeneric relationships

Precedence in the discovery of the Caucasus endemic belongs to Russian botanists of foreign origin. Representatives of the species here considered were first found in 1773 by S. G. Gmelin in Northern Iran (Gmelin, 1773) and classified by Carl Ludwig Hablitz as *Pisum ochrus* L. (Gmelin, 1774). The next finding was made by H. H. von Steven, who identified it as *Orobus formosus* (von Steven, 1812). In the first monographic summary of the Caucasus flora, *Flora Tauricacaucasica*, published between 1808 and 1819, von Steven's discovery was presented as such. Gmelin's finding was also mentioned, referred to as *Pisum ochrus* Habl. ap. S. G. Gmel. (Bieberstein, 1819). The next summary of the Caucasus vegetation reported this species as *Pisum formosum* (Stev.) Boiss. (Lipsky, 1899). However, the priority in the name *P. formosum* belonged to F. Alefeld, who first offered this combination of generic and specific names — *P. formosum* (Stev.) Alef. (Alefeld, 1861). Throughout more than two centuries of studying the species, it has undergone several name changes. Besides the above-mentioned, it was designated as *Alophotropis aucheri* (Jaub. et Spach) Grossh., *Alophotropis formosa* (von Steven) Grossh., *Pisum aucheri* Jaub.et Spach, *Vavilovia aucheri* (Jaub. et Spach) Al. Fed., and *Vicia aucheri* Boiss. (Fedorov, 1952) (see reviews by Makasheva et al., 1973; Sinjushin and Demidenko, 2010).

The last revision of the species, undertaken by A. A. Fedorov (1939), made it possible to reclassify it into a separate genus, named *Vavilovia* Fed. in honor of Acad. N. I. Vavilov. It is appropriate to mention here that this happened a year before the famous scientist was arrested.

The number of species within this genus (one or two) remained debatable for a long time, until A. A. Fedorov (1939), who had divided the genus into two species (*V. formosa* Fed. and *V. aucheri* Fed.), later changed his mind and assumed the genus monotypic (Fedorov, 1952). A. Grossheim (1952), who named the genus after one of the sections of gen. *Pisum: Alophotropis* Jaub. et Sp., also supported its division into two species*: A. formosum* and *A. aucheri*. The same opinion was shared by P. M. Zhukovsky (1971); he attributed the plant to *Alophotropis*, where perennial wild species were assembled: *P. formosum* (Stev.) Boiss. and *P. ausheri* Jaub. et Sp. The two species differed in their areas of distribution and minor features of the leaflet shape and size (*P. ausheri* had smaller leaflets). The first species occurred on the Main Caucasus Range, in the upper reach of the Kuban River, in Azerbaijan (Qusary settlement on the Qusar-chai river) and Abkhazia (on the Khojal mountain peak), while the second one was found in the Southern Trans-Caucasus: Armenia, Karabakh, Iran, and the eastern highland of Asia Minor.

A majority of the researchers who studied the species believed that it was related to peas. VIR's eminent monographers of the genus *Pisum*, L. I. Govorov (1937) and R. Kh. Makasheva (Makasheva, Drozd, Adamova and Golubev, 1973), included it in the system of the genus with the status of the species *P. formosum* (Stev.) Boiss. Similarities and differences of the genus diagnostic traits within the Fabaceae family, reported by A. A. Fedorov (1952), persuaded them that the species in question combined properties of plants from several genera: *Pisum*, *Lathyrus* and *Vicia*.

Basic differences in the biology of *P. sativum* and *P. formosum* were found, first of all, in the reproduction pattern: the first reproduces by seeds, and the second primarily by its long rhizomes; secondly, in the annual life cycle of the first species, and the perennial one of the second, although both features were not recognized as diagnostic for a leguminous genus. Meanwhile, the closeness between the two species could be implied by the susceptibility of *P. formosum* to specialized pea pathogens, traced by VIR's scientists — such as rust or *Uromyces pisi* (Pers.) Schroet.) — and blights: *Ascochyta pisi* Lib. and *Ascochyta pinodes* L. K. Jones (Yankov and Golubev, 1999). The most severely affected by pea rust were *P. formosum* plants from the Eastern Caucasus (Dagestan), which indicated the were closer to the Abyssinian pea (*P. sativum* ssp. *abyssinicum)*, a primitive cultivated form (Makasheva, 1979).

As their complex research was underway and scientific data were being accumulated, VIR's experts approved the new status of the studied plant, recognizing it as an independent genus, named *Vavilovia*. Since the release of the monographic book *Cultivated Flora. Peas* (Makasheva, 1979), this species was referred to solely as *V. formosa*.

Impressive results, clarifying the relationships within the Fabeae tribe, were obtained by immunochemical methods, widely applied in the 1970s–1980s. The essence of the method used was the analysis of individual proteins by means of their complementary bonding with similar regions of related proteins — antigenic determinants. Coincidence of all antigenic determinants in the compared proteins was expressed as a reaction of immunochemical identity (Eggi and Gavrilyuk, 1979). The more antigenic determinants the proteins of the compared species had in common, the closer they were to each other phylogenetically (Konarev, 1983).

Protein spectra of the species within the Fabeae tribe attested to a considerable remoteness of *P. sativum*

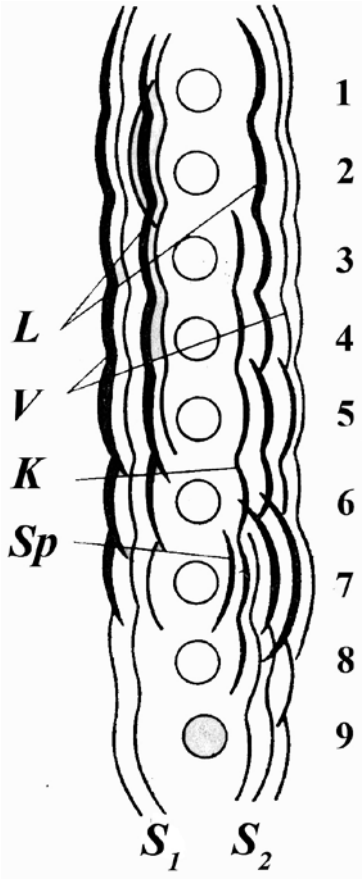

Fig. 4. Precipitation spectra for seed proteins of Sp and grasspea: L — legumin; V — vicilin; K — cathodal globulin; C — specific protein; 1 — *P. sativum* subsp. *abyssinicum*; 2 — *P. sativum* subsp. *sativum*; 3 — *P. sativum* subsp. *asiaticum*; 4 — *P. sativum* subsp. *trancaucasicum*; 5 — *P. sativum* subsp. *elatius; 6 — P. fulvum; 7 — Vavilovia formosa; 8 — Lathyrus sativus; 9 — L. aphaca; S_1* — serum for pea seed proteins; S_2 — serum for grasspea seed proteins (adapted from Tarlakovskaya, 1979).

and *V. formosa.* Their proteins had very few common components (Fig. 4). Legumin and vicilin in *V. formosa* were only partially identical with those in peas. At the same time, an interesting component was found in the cathodal area of the species' immunoelectropherogram: the so-called 'cathodal globulin', also characteristic of all the studied representatives of the Asian (*P. sativum* subsp. *asiaticum)* and Trans-Caucasian (*P. sativum* subsp. *trancaucasicum)* subspecies of *Pisum*. It was detected in seed proteins of a majority of cultivated pea (*P. sativum* subsp. *sativum)* landraces from phylogenetically ancient ecogeographic groups: Ethiopian, Mountain Anatolian, Mongolian, and Siberian. Cathodal globulin was absent in the proteins of the accessions of European origin. On this basis, cathodal globulin was recognized as a characteristic protein marker for the primitive forms. This protein was also identified in wild peas, *P. sativum* subsp. *elatius* (Bieb.) Schmalh., and in the most ancient (primitive) forms of vetch, grasspea and lentil, basically of Asian origin. Those findings suggested that the studied species was more ancient and more primitive than cultivated peas. Besides, seed proteins of *V. formosa* were found to contain an intensive component that was absent in the proteins of both *P. sativum* and wild *P. fulvum*. Significant differences in proteins were observed between *V. formosa* and *Lathyrus* spp., although they demonstrated the highest number of components in common (see Fig. 4).

All these data served as arguments in favor of an independent status of *Vavilovia*, a phylogenetically ancient genus in the authors' opinion. A suggestion was made that its ancestor had probably emerged before *Pisum* and *Lathyrus* separated into independent genera (Makasheva, 1979; Tarlakovskaya, Gavrilyuk and Makasheva, 1980).

Intraspecific differentiation

The analysis of the material delivered by VIR's collecting teams showed certain differences between the plants collected in the Caucasus and those from the Trans-Caucasus. They revealed themselves in the shape and size of leaves. The plants from Dagestan had leaflets with a subcircular base, while those from Armenia had a cuneate leaflet base and a considerably lesser size (Fig. 5, 6). These traits, in addition to the differences in the areas of distribution, were taken into account by P. M. Zhukovsky (1971) when he considered division of the section Alophotropis into the species *P. formosum* and *P. ausheri.* However, when plants from the Caucasian and Trans-Caucasian populations were grown in the same environments, they appeared indistinguishable (Golubev, 1990). This circumstance explained the tendency to regard them as ecotypes. At VIR, the terms 'Kurush' (Dagestani) and 'Gegham' (Armenian) ecotypes were in use (Golubev, 1990).

Studying protein spectra of the plants belonging to those ecotypes added some new data to intraspecific differentiation. Sharp differences were revealed for the two populations — much more significant than between plant forms belonging to different *Pisum* subspecies, such as, for example, *P. sativum* subsp. *elatius* and *P. sativum* subsp. *sativum*.

Some researchers suggested a possibility that there were two varieties (Govorov, 1937) or even species (Zhukovsky, 1964). With this in view, similarities were found between the proteins of the Dagestani population of *V. formosa* and those of vetch, and between the proteins of the Armenian population of the species and those of grasspea (Fig. 7).

All those research efforts once more led to the conclusion that the studied species was an integral, combining in itself the traits of pea, grasspea and vetch (Tarlakovskaya, Gavrilyuk and Makasheva, 1980).

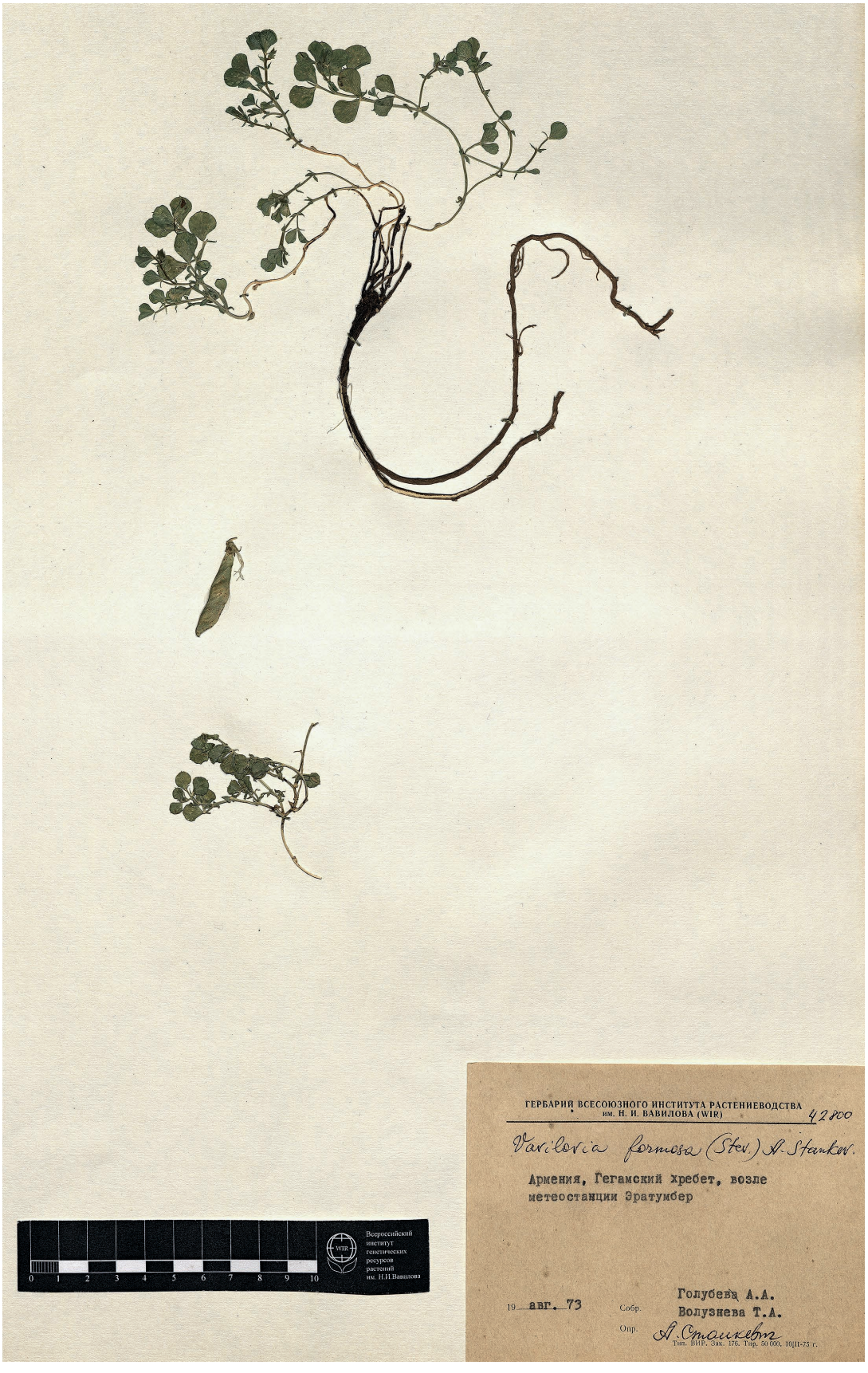

Fig. 5. *V. formosa* from the Gegham Ridge, Armenia, collected near the Eratumber weather station, 3100 MASL, on August 19, 1973 (WIR).

Fig. 6. *V. formosa* from the Ragdan-chai gorge, Dagestan, 2660–2700 MASL, collected on August 31, 1973 (WIR).

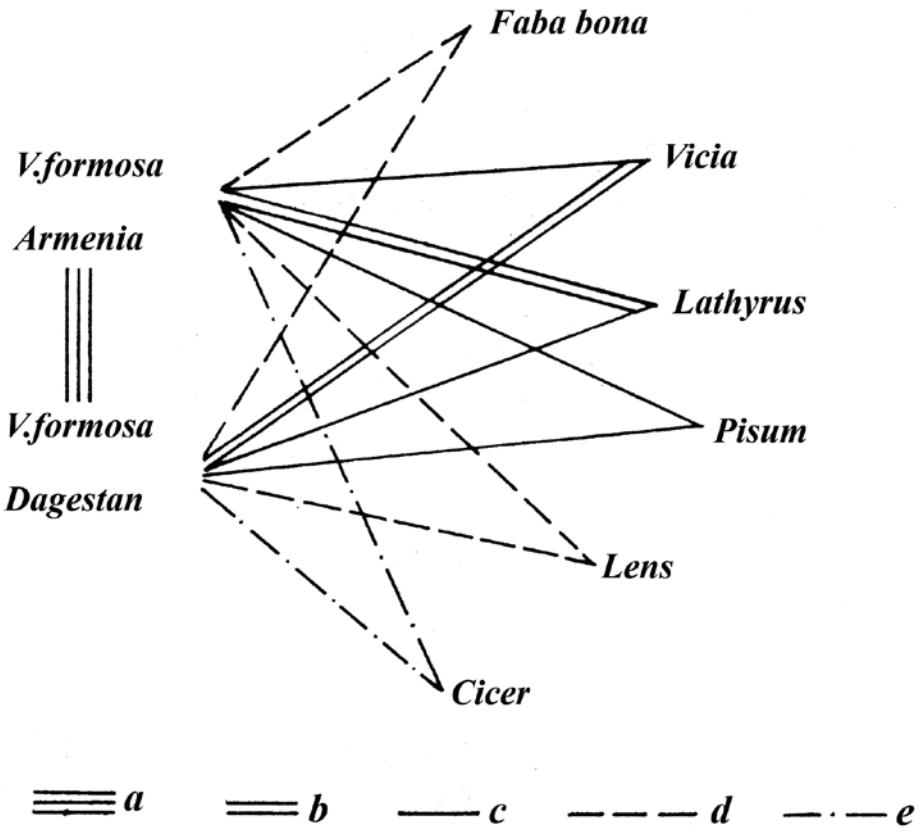

Fig. 7. A diagrammatic image showing degrees of relationships between *Vavilovia formosa* and other Fabeae according to the data of immunochemical analysis of seed proteins. The numbers of antigenic proteins in common: a — 11, b — 10, c — 9–8, d — 7–6, e — 5–4 (adapted from Tarlakovskaya, Gavrilyuk and Makasheva, 1980).

Remote crossing

The ensuing experiments on *V. formosa* hybridization — the only ones known for their successful results — were also accomplished at VIR.

In 1974–1983, the delivered plants — those that had been dug out with roots and reproduced from seeds — were grown in the field, greenhouse, and climate chamber. For the first time, it became possible to invoke flowering in perennial pea plants, but no seed reproductions were obtained. The details of changing the plant growing regimes are reported in the publication by A. A. Golubev (1990).

The first attempts of remote crosses involving *V. formosa* were made as early as 1978. Plants of pea, grasspea and vetch were delivered from VIR (Leningrad) to Dagestan, where their pollen was used to pollinate the endemic's flowers in its natural habitats (vicinities of Kurush Village). Those attempts were a failure. Flowers of *V. formosa* were shipped to VIR to be used as the paternal component in crosses. It appeared that the pollen, when preserved under dry conditions at 5–6 °C, retained its viability for 28 days (Golubev, 1990).

Artificial intraspecific transpollination failed to yield visible results, which is one more piece of evidence of the need for cross-pollination. Mature pods and seeds were produced for the first time in the autumn and winter of 1987–1988.

Numerous attempts to cross *V. formosa* with the cultivated pea in a greenhouse (Pushkin Town, Leningrad Province) resulted in obtaining several normally developed seeds from the combination *V. formosa* × *P. sativum*. Out of those, only one seed succeeded in producing a true hybrid plant on a culture medium that facilitated morphogenesis. The plant had several stems or basal branches, with long internodes and without the lateral branches typical of *V. formosa*. Its leaves were compound, with one pair of leaflets, plus a third smaller leaflet instead of the rachis, characteristic of the species in question. As a result, the leaves became similar to those of clover or alfalfa. That solitary hybrid plant ever to have been obtained failed to enter the generative phase and ultimately died from chlorosis (Golubev, 1990).

The reverse combination *P. sativum* × *V. formosa* also yielded one hybrid plant, grown without any additional manipulations. The plant manifested signs of heterosis: it was much taller than both parents, produced numerous basal and lateral branches, and developed flowers as well as five pods. However, no viable mature seeds were produced (Golubev, 1990).

Aggregately, those experiments visibly demonstrated the reproductive isolation of *V. formosa* from other species of the Fabeae tribe.

VIR's investigations of the 1970s–1980s in the context of modern achievements

Thus, from 1960 through the late 1980s, VIR undertook pioneering research into the ecology, karyology and anatomy of the Fabeae tribe, identifying phylogenetic relationships therein with immunochemical methods, and performed interspecific crosses between *V. formosa* and other members of the tribe. In the early 20th century, especially with the development of molecular-genetic and molecular-phylogenetic research, interest in the Caucasian endemic showed abruptly increased.

The intraspecific differentiation was studied by A. Sinjushin and N. Demidenko (2010); A. Sinjushin and A. Belyakova (2010); and P. Smykal et al. (2017). Heterophylly was observed in all analyzed plant samples, a phenomenon present across the *Fabeae* tribe. In most samples the coefficient of variation (CV) for the leaflet area exceeded 50 %. As in the previous studies, a certain geographic regularity was found in the distribution of leaf sizes: plants of northern populations (Kuban, Dagestan, etc.) had a larger leaf area, compared with southern ones (Turkey, Lebanon, etc.). Later, a significant positive correlation between the geographic latitude and the leaf area ($\rho\rho = 0.527$, $p < 0.05$) was found (Smykal et al., 2017).

In his works dedicated to the altitudinal variability of plants, N. Vavilov mentioned that "…mountainous areas…store a great diversity of ecotypes" (Vavilov, 1965, p. 125). In their initial observations, even the first collecting missions launched by VIR noticed differences in the plant habit of *V. formosa* samples occurring on different altitudes of the same mountain: at 2700–2800 MASL (a stony, coarsely crushed, less mobile substrate), the plants were 5–10 cm high, with smaller leaflets; but on a more mobile finely crushed stone substrate (2500–2700 MASL), plant height reached 15 cm and leaflets were larger (Makasheva, Drozd, Adamova and Golubev, 1973). Such differences are seen as typical evidence of intraspecific ecotypical differentiation.

The differences that had evolved as a result of variable environmental conditions in plant habitats revealed themselves in the process of studying the DNA polymorphism of *V. formosa* using the RAPD technique. It should be mentioned that quite a few researchers reported on the ability of the applied marker to reveal the adaptive ecogeographic differentiation (Nevo, Baum, Beiles and Johnson, 1998; Zhao et al., 2006; Sharmila, Akilandeswari, Ramya and Mownika, 2019; etc.). As a result of analysis, a clade of *V. formosa* was formed together with its two subclades, complying with two regions of the plant's origin: Armenia–Nakhichivan and Kuban–Dagestan (Sinjushin and Demidenko, 2010). The authors of that publication, following L. Govorov (1937), assumed that this species may be divided into two varieties or subspecies. According to the concept of E. N. Sinskaya (1948), they could be regarded as two geographic ecotypes, also interpretable as subspecies.

Smaller sizes of leaves, similarly to the size of plants themselves, in Trans-Caucasian populations might be additionally explained by more severe environments of the Gegham Ridge, compared with the northern slopes of the Main Caucasus Range and the environments of Dagestan. While analyzing the *V. formosa* collection sites in the Caucasus *sensu stricto*, it becomes obvious that in Armenia the localities where its plants have been found are situated much higher than on the northern slopes of the Main Caucasus. In the western part of the Caucasus Range, *V. formosa* was collected at the altitudes from 2500 up to 3100 MASL; the lowest collecting sites were recorded for the central Caucasus (2021–2850 MASL), and the highest in its eastern part (2500–3500 MASL). A distinctive feature of the collecting in Armenia was that all known populations were found at high altitudes, never lower than 3000 MASL, on glacier-covered mountains (Vishnyakova et al., 2016).

Molecular-phylogenetic research of recent years, undertaken worldwide by many laboratories, contributed new, sometimes unexpected data to the understanding of the Fabeae tribe's phylogenesis. After studying fragments of three phylogenetically informative regions within the tribe: chloroplast maturase K, trn L-F, and trn S-G, along with the internal transcribed spacer (ITS) region of nuclear DNA, the species *V. formosa* stood out as a separate clade within the larger clade, consisting of *Pisum*, *Vavilovia* and *Lathyrus*, neighboring with *Vicia*. The results testify to the monophyly of *Pisum* and *Vavilovia*, and show that they form a monophyletic pairing: the *Pisum* — *Vavilovia* clade is sister to most of *Lathyrus*, and all are nested within *Vicia* (Smykal et al., 2010).

The monophyly of *Pisum* and *Vavilovia* was confirmed by the research into the Fabeae tribe using a complex of morphological traits and a set of molecular data. The best, maximum-likelihood tree, based on combined data on the polymorphism of chloroplast and nuclear DNA, was developed for 470 plant samples representing 262 species and 7 external groups (Schaefer, 2012). The *Pisum*–*Vavilovia* branch topologically appeared within the genus *Lathyrus*; the *Lens* branch — within the genus *Vicia*. The divergence of the genera *Pisum* and *Vavilovia* is supposed to have occurred 9.8–4.8 million years ago.

It is quite obvious that the modern molecular methods, with their high resolving capacity, are a reliable tool for phylogenesis. Therefore, the hypothesis about the divergence of the Fabeae genera, formulated 40 years ago by VIR's researchers (Makasheva, 1979; Tarlakovskaya, Gavrilyuk and Makasheva, 1980), is now of historical

value only. A similar situation is not infrequent when the results of conventional, essentially based on a limited set of traits, and molecular phylogeneses are compared. It can be convincingly explained by the remarkable progress in the development of available research techniques.

It should be mentioned that those data of molecular phylogenesis (Schaefer, 2012) initiated a revision of the Fabeae tribe classification, proposed by French scientists P. Coulot and F. Rabaute (Coulot and Rabaute, 2016). Their nomenclatural interpretation, however, is not always in line with the existing International Code of Nomenclature for Algae, Fungi and Plants (International Code …, 2012) and has not yet been widely discussed (Kosterin, 2017).

Nevertheless, even within the boundaries of new taxonomic constructs, *V. formosa* retains the status of an independent species.

Conclusion

This paper summarized the research cycle undertaken at VIR to study the species *Vavilovia formosa*, a wild crop relative listed in the Red Data Books of the USSR, Russia, a number of Russian federal subjects, and several neighbor countries. All the studies, mostly conducted in the 1960s–1980s, were innovative for that time. Modern research, carried out in a number of laboratories worldwide, contributed some new data to the understanding of the species' biology. Thanks to modern techniques with much higher resolving capacity, this research made it possible to develop detailed phylogenetic constructs, which confirmed the independent status of *V. formosa* as a species, and identified its place within the system of the Fabeae tribe.

VIR's research cycle was interrupted for many years by certain events that happened in the Russian economy in the 1990s and resulted in closing research laboratories and programs at VIR. By now, a new generation of scientists has emerged to replace the older one, whose representatives made an extremely valuable contribution to the knowledge of the biology of this species as a relic of the floristic kingdom, which is still a reserve for future discoveries. Searching for the species' habitats and ways of its conservation both ex situ and in situ remains a crucial task. Research into *V. formosa* has fundamental and practical value for plant breeding, genetics, genomics, metabolomics, symbiogenetics, evolution, and new scientific research trends, such as reverse plant breeding and plant neodomestication.

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References

Abramova, L. I. 1971. The number of chromosomes and some karyotype features of *Pisum formosum* (Stev.) Boiss. *Bullitin of Applied Bototany, Genetics and Plant Breeding* 45:240–243.

Alefeld, F. 1861. Ueber Viceen. "Bonplandia". Jahrgang. 15(9):236–237.

Akopian, J., Sarukhanyan, N., Gabrielyan, I., Vanyan, A., Mikić, A., Smýkal, P., Kenicer, G., Vishnyakova, M., Sinjushin, A., Demidenko, N., and Ambrose, M. 2010. Reports on establishing an ex situ site for 'beautiful' vavilovia (*Vavilovia formosa*) in Armenia. *Genetic Resources and Crop Evolution* 57:1127–1134. https://doi.org/10.1007/s10722-010-9606-0

Akopian, J., Ghukasyan, A., Hovakimyan, Zh., Martirosyan, L., and Zaroyan, G. 2019. Experience of wild perennial pea *Vavilovia formosa* cultivation in the botanical gardens of Armenia. Yerevan, Institute of Botany after A. Takhtajyan of NAS RA. 14 p.

Astamirova, M. A-M. 2011. The genesis of the Upper Alpine rock-scree flora of the Eastern Caucasus. Methods of analytical floristics and problems of florogenesis. Materialy pervoj mezhdunarodnoj nauchnoj i prakticheskoj konferencii (Astrakhan, August 7–10, 2011), pp. 131–139.

Astamirova, M. A.-M., Abdurzakova, A. C., Magomadova, R. S., Umaeva, A. M., Omarhadzhieva, F. S., Hasueva, V. A., Dudagova, E. Sh., and Shahgirieva, Z. I. 2011. History of the study of rock-scree flora and vegetation of the Upper Alpine belt of the Eastern Caucasus. *Nauchnaya gazeta Belgorodskogo gosudarstvennogo universiteta. Seriya estestvennye nauki* 15–2(104):179–188. (In Russian)

Bieberstein, F. M. 1819. Flora Taurico-Caucasica exhibens stripes phaenogamas in Chersoneso Taurica et regionibus Caucasicis sponte crescentes. *Charcoviae* 3:654.

Coulot, P. and Rabaute, P. 2016. Monographie de Leguminosae de France. Tome 4. Tribus des Fabeae, des Cicereae et des Genisteae. *Bulletin de la Société Botanique du Centre-Ouest* 46:1–902.

Eggi, E. E. and Gavrilyuk, I. P. 1979. Immunochemical specificity of the main globulins in seeds of legumes. *Proceedings of Applied Botany, Genetics and Plant Breeding* 63(3):145–156. (In Russian)

Fedorov, An. A. 1939. Wild high-mountain peas of Caucasus. *Trudy biologicheskogo instituta Armyanskoj SSR* 1:39–79. (In Russian)

Fedorov, An. A. 1952. Supplement. In: Flora of the Caucasus. Volume 5. (Grossheim, A. A., ed.) Academy of Science of the USSR, St. Petersburg. (In Russian)

Gmelin, S. G. 1774. Reisedurch Russland zur Untersuchung der drey Natur-Reiche. 4. Kanserlichen Academie der Wissenschaften, St. Petersburg.

Golubev, A. A. 1990. Habitats, collection, cultivation and hybridization of *Vavilovia formosa*. *Bulletin of Applied Botany, Genetics and Plant Breeding* 135:67–75. (In Russian)

Govorov, L. I. 1937. Pea. In: Flora of cultivated plants. IV. Grain Leguminosae. (Vavilov, N. I., Wulff, E. V., eds). M.-L., pp. 231–338. (In Russian)

Grossheim, A. A. 1952. Genera *Vavilovia*. In: Flora of the Caucasus. Volume 5. (Grossheim, A. A., ed.) Academy of Science of the USSR, St. Petersburg. (In Russian)

International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). 2012. Oberreifenberg: Koeltz Scientific Books.

Kharadze, A. L. 1960. Endemic hemikserophilic element high mountains of Great Caucasus. *Problems of botany* 5:115–126. (In Russian)

Konarev, V. G. 1983. Plant proteins as genetic markers. Moscow, Kolos. 319 p. (In Russian)

Kosterin, O. E. 2017. Abyssinian pea (*Pisum schaeferi* Kosterin pro *Pisum abyssinicum* A. Br.) — a problematic taxon. *Vavilov Journal of Genetics and Breeding* 21(2):158–169. https://doi.org/10.18699/VJ17.234 (In Russian)

Levitskyi, G. A. 1930. Investigation of chromosome morphology. *Proceedings of the USSR Congress of genetics, plant- and animal breeding* (Leningrad, 10–16 January 1929). (In Russian)

Makasheva, R. Kh., Drozd, A. M., Adamova, O. P., and Golubev, A. A. 1973. Perennial pea. *Bulletin of Applied Botany, Genetics and Plant Breeding* 51:44–56. (In Russian)

Makasheva, R. Kh. 1979. Pea. Flora of Cultivated Plants IV, Grain Legumes IV, Kolos, St. Petersburg. 321 p. (In Russian)

Mikić, A., Smýkal, P., Kenicer, G., Vishnyakova, M., Akopian, J., Sarukhanyan, N., Gabrielyan, I., Vanyan, A., Toker, C., Ćupina, B., Ambrose, M., Mihailović, V., and Ellis, N. 2009. A revival of the research on beautiful Vavilovia (*Vavilovia formosa* syn. *Pisum formosum*). *Pisum Genetics* 41:34–39.

Mikić, A., Smýkal, P., Kenicer, G., Vishnyakova, M., Sarukhanyan, N., Akopian, J., Vanyan, A., Gabrielyan, I., Smýkalová, I., Sherbakova, E., Zorić, L., Atlagić, J., Zeremski-Škorić, T., Ćupina, B., Krstić, Đ., Jajić, I., Antanasović, S., Đorđević, V., Mihailović, V., Ivanov, A., Ochatt, S., Ambrose, M. 2013. The bicentenary of the research on 'beautiful' vavilovia (*Vavilovia formosa*), a legume crop wild relative with taxonomic and agronomic potential. *Botanical Journal of Linnean Society* 172:524–531. https://doi.org/10.1111/boj.12060

Murtazaliev, R. A., Musaev, M. A., Burlyaeva, M. O., Huseynov, Z. A., et al. 2012. *Vavilovia formosa* and *Cicer minutum* (Fabaceae) in Dagestan. *Botanical Journal* 97:961–965. https://doi.org/10.1134/S1234567812070087 (In Russian)

Nevo, E., Baum, B., Beiles, A., and Johnson, D. A. 1998. Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. *Genetic Resources and Crop Evolution* 45:151–159. https://doi.org/10.1023/A:1008616923427

Ochatt, S., Conreux, C., Smýkalová, I., Smýkal, P., and Mikić, A. 2016. Developing biotechnology tools for 'beautiful' vavilovia (*Vavilovia formosa*), a legume crop wild relative with taxonomic and agronomic potential. *Plant Cell, Tissue and Organ Culture* 127(3):637–648. https://doi.org/10.1007/s11240-016-1148-5

Oskoueiyan, R., Kazempour, O. S., Maassoumi, A. A., Nejadsattari, T., and Mozaffarian, V. 2010. Phylogenetic status of *Vavilovia formosa* (Fabaceae-Fabeae) based on nrDNA ITS and cpDNA sequences. *Biochemical Systematics and Ecology* 38:313–319. https://doi.org/10.1016/j.bse.2010.01.011

Petrova, M. V. 1973. Anatomical characteristics of *Pisum formosum* (Stev.) Boiss. *Bulletin of Applied Botany, Genetics and Plant Breeding* 54(1):126–134. (In Russian)

Prima, V. M. 1974. Subnival flora of the Eastern Caucasus: its composition, ecological, biological, and geographical analysis. Flora and vegetation of the North Caucasus: Proceeding articles. Ordzhonikidze, pp. 46–69. (In Russian)

Report about the expedition to Caucasus, 1972–1973. The archive of the Introduction department of Vavilov Institute of Plant Genetic Resources. (In Russian)

Report about the expedition to Caucasus, 1975. The archive of the Introduction department of Vavilov Institute of Plant Genetic Resources. (In Russian)

Safronova, V. I., Kimeklis, A. K., Chizhevskaya, E. P., Belimov, A. A., Andronov, E. E., Pinaev, A. G., Chizhevskaya, E. P., , Pukhaev, A. R., Popov, K. P., Willems, A., and Tikhonovich, I. A. 2014. Genetic diversity of rhizobia isolated from nodules of the relic species *Vavilovia Formosa* (Stev.) Fed. *Antonie van Leeuwenhoek* 105:389–399. https://doi.org/10.1007/s10482-013-0089-9

Safronova, V. I., Kuznetsova, I. G., Sazanova, A. L., Kimeklis, A. K., Belimov, A. A., Andronov, E. E., Pinaev, A. G., Pukhaev, A. R., Popov, K. P., and Tikhonovich, I. A. 2015. *Bosea vaviloviae* sp. nov., a new species of slow-growing rhizobia isolated from nodules of the relict species *Vavilovia Formosa* (Stev.) Fed. *Antonie van Leeuwenhoek* 107:911–920. https://doi.org/10.1007/s10482-015-0383-9

Schaefer, H., Hechenleitner, P., Santos-Guerra, A., Menezes de Sequeira, M., Pennington, R. T., Kenicer, G., and Carine, M. A. 2012. Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. *BMC Evolutionary Biology* 12:250. https://doi.org/10.1186/1471-2148-12-250

Sharmila, S., Akilandeswari, D., Ramya, E. K., and Mownika, S. 2019. Ecology and the RAPD techniques used to assess the genetic diversity in *Pterolobium hexapetalum*, a scrambling medicinal shrub in Maruthamalai and Chennimalai hills, the Western Ghats. *International Journal of Pharmaceutical Sciences Review and Research* 11(3):72–77. https://doi.org/10.22159/ijpps.2019v11i3.30983

Sinskaya, E. N. 1948. The dynamics of the species. Moscow-Leningrad, 526 p. (In Russian)

Sinjushin, A. A. and Demidenko, N. V. 2010. *Vavilovia formosa* (Stev.) Fed. (Fabeae, Fabaceae) on Meyen's 'panel with a multitude of lamps. *Wulfenia* 17:45–57.

Sinjushin, A. A. and Belyakova, A. S. 2010. On intraspecific variation of *Vavilovia formosa* (Stev.) Fed. (= *Pisum formosum* (Stev.) Alef.: Fabeae). *Pisum genetics* 42:31–34.

Smýkal, P., Kenicer, G., Flavell, A. J., Corander, J., Kosterin, O., Redden, R. J., Ford, R., Coyne, C. J., Maxted, N., Ambrose, M. J., and Ellis, N. T. H. 2011. Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. *Plant Genetic Resources* 9(1):4–18. https://doi.org/10.1017/S147926211000033x

Smýkal, P., Chaloupská, M., Bariotakis, M., Marečková, L., Sinjushin, A., Gabrielyan, I., Akopian, J., Toker, C., Kenicer, G., Kitner, M., and Pirintsos, S. 2017. Spatial patterns and intraspecific diversity of the glacial relict legume species *Vavilovia Formosa* (Stev.) Fed. in Eurasia. *Plant Systematics and Evolution* 303:267–282. https://doi.org/10.1007/s00606-016-1368-5

Tarlakovskaya, A. M. 1975. The position of *Pisum formosum* (Stev.) Alef. in tribe Fabeae Bronn. on the basis of immuno-chemical protein analysis. *Research Bulletin of the N. I. Vavilov Institute of Plant Industry* 47:32–34. (In Russian)

Tarlakovskaya, A. M. 1979. Seed proteins as species and genera markers in tribe Fabeae. *Research Bulletin of the N. I. Vavilov Institute of Plant Industry* 92:53–58. (In Russian)

Tarlakovskaya, A. M., Gavrilyuk, I. P., and Makasheva, R. H. 1980. Immuno-chemical differentiation of *Vavilovia formosa* Fed. from different cites of habitat. *Research Bul-*

letin of the N. I. Vavilov Institute of Plant Industry 97:29–34. (In Russian)

The Red Book of the Russian Federation. Plants and fungi. 2008. Association of Scientific Publications KMK, Moscow. (In Russian)

The Red Book of the USSR. Rare and endangered species of animals and plants. 1984. Volume 2. Moscow. (In Russian)

Vavilov, N. I. 1965. Geographic variability of plants. Selected works. Moscow-Leningrad, pp. 120–126. (In Russian)

Vishnyakova, M. A., Burlyaeva, M. O., Seferova, I. V., Bagmet, L. V., and Semenov, V. A. 2013. Expedition collections of the tribe Fabeae representatives in the Russian Federation and the adjacent territories of the North Caucasus. *Proceedings of Applied Botany, Genetics and Plant Breeding* 172:82–86. (In Russian)

Vishnyakova, M., Burlyaeva, M., Akopian, J., Murtazaliev, R., and Mikić, A. 2015. Reviewing and updating the detected locations of beautiful vavilovia (*Vavilovia formosa*) on the Caucasus *sensu stricto*. *Genetic Resources and Plant Evolution* 63(7):1085–1102. https://doi.org/10.1007/s10722-016-0440-x

Von Steven, C. 1812. Catalogue des Plantes. *Mémoires de la Société des naturalistes de Moscou* 3:244–270.

Von Steven, C. 1812–1813. Stirpes rariores in itinere Caucasico. *Mémoires de la Société des naturalistes de Moscou* 4.

Yan'kov, I. and Golubev, A. A. 1999. About the taxonomical status of *Vavilovia formosa* based on the results of crossability and susceptibility to specialized pathogens. CD of the XVI International Botanical Congress, St. Louis, US: 5655.

Zhukovskyi, P. M. 1971. Cultural plants and their relatives. 3-rd edition. Publishing house "Kolos", Leningrad. 751 p. (In Russian)

Zhao, N., Gao, Yu., Wang, J., Ren, A., and Xu, H. 2006. RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. *Acta Ecologica Sinica* 26(5):1312–1318. https://doi.org/10.1016/S1872-2032(06)60023-1